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TITLE: The Blood-testis-barrier and Male Sexual Dysfunction Following Spinal Cord Injury

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14. ABSTRACT A majority of males exhibit a profound loss of fertility following spinal cord injury. While the mechanisms underlying this loss have been discussed for decades, recently my lab discovered that spinal trauma produces a significant loss in integrity of the blood-testis-barrier; a protective multi-cellular structure that maintains immune privilege of the highly-antigenic sperm and sperm cell-containing compartments within the testis. We also demonstrated that once failed, the BTB remains permeable, essentially for the life of the subject. The goal of our proposal has been two-fold: 1) to develop a greater understanding of the molecular, biochemical and structural pathologies underlying BTB breakdown post-SCI, and 2) to determine whether a novel therapeutic, recently identified in our laboratory, can help preserve BTB integrity when introduced during the acute phase of SCI using a clinically-relevant rat spinal contusion model. We have found that the drug, licofelone, preserves blood-spinal cord barrier integrity and enhances locomotor function in rats when given early following injury. During this first year, we have performed all planned spinal cord injuries (24 hour out to 90 day time points); collected testis tissues and have sent samples out for metabolomic analysis and gene array studies. Remaining tissues are banked for Western blot and histological assessments that will be determined by the outcomes generated via the metabolomic and array studies.					
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Introduction:

Males who have received a spinal cord injury (SCI) face a lifetime of sensory and motor deficits. In addition to these well-described pathological outcomes, a majority of men will also experience a profound loss of fertility. This should be clearly understood to be separate from SCI-dependent erectile dysfunction that is due to a loss of neural input into the male sexual organs. SCI-dependent male infertility is characterized by a significant reduction in numbers and quality of functional sperm. The mechanism(s) underlying this deficit has previously been unknown. My laboratory has explored the effects of spinal trauma on tissues that exhibit “barrier” properties, or properties in which specialized tissues regulate the flow of materials from the blood stream into compartments throughout the body that are “immune privileged”. Our focus has been on the blood-spinal cord-barrier (BSCB) and how trauma collapses this important spinal vascular specialization; producing an environment that encourages long-term inflammatory conditions. We recently asked whether spinal trauma had any effect on the blood-testis-barrier (BTB), a specialized set of cellular structures located within the testis which protects sperm (immature through mature) as well as sperm precursor/stem cells from the immune system. We reported that a contusive injury to the rat spinal cord causes a profound and sustained loss of BTB integrity; resulting in the formation of both inflammatory and pro-oxidative conditions within the sperm-producing compartments. In addition, we detected the loss of structural elements that comprise the BTB as well as significant cell death and immune cell infiltration. The goal of this project is to: 1) further elaborate the early and long-term biochemical, molecular and structural deficits to the BTB elicited by spinal trauma, and 2) determine whether these pathological changes can be prevented or at least minimized by pharmacological modulation. We have recently found that a novel anti-inflammatory drug, licofelone, provided significant protection to the BSCB when administered to rats orally during the acute phase of SCI. Licofelone is a first generation anti-inflammatory drug that targets BOTH cyclooxygenase AND 5-lipoxygenase pathways of arachidonic acid metabolism; the two main pathways used to generate arachidonic acid-derived pro-inflammatory compounds (prostaglandins and leukotrienes, respectively). In the second aim of the current project, we will be determining whether acute licofelone treatment preserves BTB integrity and reduces inflammation and cell death within the testis. As of November 2013, we have completed all proposed surgeries to be performed under the initial aim of the study. Testis tissues have been sent off for an assessment of metabolic changes (through Metabolon, Inc) as well as differences in gene expression resulting from SCI from early to chronic time points (through the gene array core facility run by my colleague and Co-PI, Dr. David Loose). In addition, other testis samples have been collected and frozen for additional analysis of protein expression via Western blot and immunohistochemical methods. Our goal is to identify specific protein targets for these analyses based on the metabolic and gene expression data. Metabolomic data is anticipated by the end of November/early December 2013 with the gene array data anticipated in early January, 2014.

Body:

Despite this being a year summary of progress/listing of results, I can note only the following: After attaining all of the necessary approvals to conduct the study, I also identified an MD/PhD student by the name of Ryan Fortune who has taken on this project as his thesis work. Ryan is a first lieutenant in the Army Reserve and has expressed a profound interest in working in the area of trauma surgery; particularly in regards to CNS trauma. Ryan received extensive training from me in the adult rat spinal contusion injury model as well as all subsequent animal care procedures. He has also learned how to collect tissues and store them during the terminal endstage procedures. Under my direction, Ryan has been responsible for performing all animal surgeries listed under the first specific aim (see below). In addition, Ryan has been responsible for preparing all tissues for subsequent processing and analysis by Metabolon, Inc (metabolism studies) and gene expression studies (through the microarray core operated by Dr. David Loose, Co-PI of this study and Director of the Molecular Core within the Department of Integrative Biology and Pharmacology.

Specific Aim 1: Explore the molecular, biochemical and structural changes that occur to the BTB as a function of time following the delivery of a clinically-relevant spinal contusion injury.

Ryan has been responsible for performing the surgeries and subsequent animal care for the following groups:

Groups:

Naïve (uninjured)

Sham (laminectomy but no contusion)

Contusion

Time points post-SCI:

24 hours, 72 hours, 28 days and 90 days

Outcome measures: 1) metabolomic analysis, 2) microarray analysis, 3) Western blot studies, 4) immunohistopathology

Number of animals per group per time point: 16. Of this 16 per group per time point, 8 were sacrificed using a transcardial flush with saline with one testis per rat collected for Western blot analyses and the other testis collected for immunohistopathology. These testis were frozen and stored at -80 degrees. The other 8 animals per group per time point were NOT perfused. They were deeply anesthetized and decapitated with tissues quickly removed to reduce time-dependent breakdown in metabolic compounds and mRNA. Testes from these groups were processed for subsequent transport to Metabolon, Inc for their metabolic assessments while the paired testis from each subject was prepared for microarray analysis through our colleague, Dr. Loose's Core.

Total number of animals used in Aim 1: 176

Tissues have been sent off for both metabolomic and gene array studies. We anticipate receiving the metabolomic data by the end of November, 2013 and the gene array data by January, 2014. These data will be used to identify and narrow protein targets to be examined in the banked, frozen tissues via Western blot and immunohistochemical analyses. We anticipate providing our initial report on the metabolomic and gene array studies in the next quarterly report in early January, 2014.

Specific Aim 2: Determine whether treatment with the new generation anti-inflammatory drug, Licofelone, can protect BTB integrity and enhance germ cell and sperm viability over time following SCI.

We are preparing to initiate Aim 2 and the subsequent assessment of licofelone as a novel treatment for the preservation of BTB integrity following SCI.

At this point, we have not encountered any problems that would negatively impact completion of the studies by the end of the project period.

Key Research Accomplishments:

- 1) completion of all animal surgeries/tissue collections described in Aim 1.
- 2) Tissues have been forwarded on to Metabolon, Inc. and the gene array core administered by my Co-PI and colleague, Dr. David Loose for subsequent analyses. Metabolomic analyses should be sent to us by the end of November, 2013 and gene array studies by January 2014.
- 3) Initiating Aim 2.

Reportable Outcomes:

We anticipate the first reportable data outcomes in the next Quarterly Report.

I have indicated above that Lt. Ryan Fortune has entered my lab as an MD/PhD student at UT-Health. He has chosen this project as his PhD thesis project. In addition, he has successfully achieved funding for his stipend through an NIH-sponsored T32 training grant through UT-Health's Center for Clinical and Translational Sciences. As a result, we have not had to acquire an originally-requested technician for this study.

Conclusions:

We do not yet have reportable data upon which to draw conclusions.

The blood-testis-barrier and male sexual dysfunction following spinal cord injury

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PI: Raymond J. Grill

Org: UT-Health

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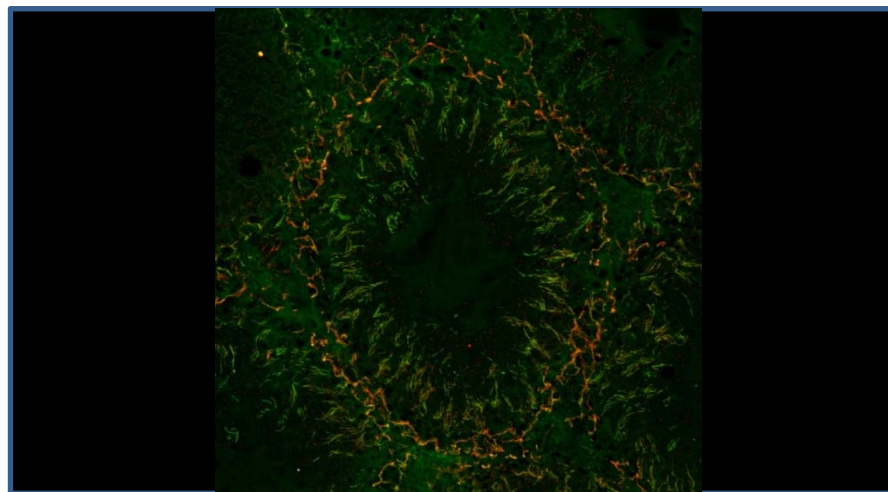


Study/Product Aim(s)

- **Specific Aim 1:** Explore the molecular, biochemical and structural changes that occur to the BTB as a function of time following the delivery of a clinically-relevant spinal contusion injury.
- **Specific Aim 2:** Determine whether treatment with the new generation anti-inflammatory drug, Licofelone, can protect BTB integrity and enhance germ cell and sperm viability over time following SCI.

Approach

Utilize a clinically-relevant rat spinal contusion model of injury to assess the early and long-term effects on the blood-testis-barrier as a mechanism underlying male infertility following spinal cord injury.



Accomplishment: Localization of occludin, a tight junction protein that contributes to the properties of the BTB, in a seminiferous tubule within the testis..

Timeline and Cost

Activities	CY	12/13	13/14
Performed all the surgeries, collected all samples and sent tissues to Metabolon and UT-Houston Microarray Core for analysis (Aim 1)			
Have begun the surgeries required for Aim 2.			
Estimated Budget (\$K)		\$257,812	\$279,628

Updated: (03/05/2014)

Goals/Milestones (Example)

CY12/13 Goal – Initiate Aim 1 of project

- ☒ Gain institutional approvals for all aspects of project
- ☐ Identify/hire individual to assist with project ☒
- ☐ train individual in surgical procedure, animal care ☒
- ☐ perform all animal surgeries outlined in Aim 1 ☒
- ☐ collect, store tissues; prepare samples and send out for metabolomic and gene array studies ☒

CY13/14 Goal – Initiate Aim 2

- ☐ Analyze all data generated from Aim 1 to identify SCI-induced targets in testis tissues.
- ☐ Perform all surgeries/treatment studies
- ☐ collect and process tissues
- ☐ analyze tissues
- ☐ Determine subsequent goals

Budget Expenditure to Date

Projected Expenditure: \$257,812

Actual Expenditure: \$99,240